ZOOLOGICAL SCIENCE 10: 653–660 (1993)

Production of Normal↔Macular Mouse Chimeras: The Presence of Critical Tissue in the Macular Mutant Mouse, a Model of Menkes' kinky Hair Disease

Shinsaku Nakagawa*[1], Ryuji Adachi[1], Masaharu Miyake[1], Takao Hama[1], Keiichi Tanaka[2] and Tadanori Mayumi[2]

[1]*Faculty of Pharmaceutical Sciences, Kobe Gakuin University, Nishi-ku, Kobe 651-21 and* [2]*Faculty of Pharmaceutical Sciences, Osaka University, Suita, Osaka 565, Japan*

ABSTRACT—The macular mouse (Mo^{ml}) is a murine model of the Menkes' kinky hair disease. The Mo^{ml} allele is a recessive lethal mutation. Mo^{ml} mice die about 15 days after birth, exhibiting behavior of some neurological disorders without evidence of a connective tissue abnormality. A multitude of morphologic abnormalities have been described in hemizygotes (Mo^{ml}/Y) and homozygotes (Mo^{ml}/Mo^{ml}). As an approach to determine the critical tissue in the macular mutant mouse, we produced chimeras from macular mutant and normal mouse embryos. Thirteen chimeras were obtained, and 11 of these chimeras survived without copper treatment. In surviving chimeras, normal mouse cells were present in all tissues. However, 2 chimeras died at days 16 and 22 after birth, respectively. The normal mouse cells were not distributed in the liver of both chimeras. The copper concentration in the liver of both chimeras were significantly low in the same manner as the Mo^{ml}/Y and Mo^{ml}/Mo^{ml} mouse. Our findings provide important evidence in the search for "critical tissue" in the macular mouse.

INTRODUCTION

Menkes' kinky hair disease in humans is a well established genetic entity, inherited as an X-linked recessive, and affecting the central nervous system, skin, hair, blood vessels and bones. Its metabolic defect is considered to be due to a failure in copper homeostasis in the body in which copper accumulates in some organs including the intestinal mucosa and kidney, but is deficient in others including the brain and liver [1, 2, 4].

The macular mutation (Mo^{ml}) found by Nishimura [11] is an allele at the X-linked Mottled (*Mo*) locus. The mutant mouse has many clinical and biochemical similarities to Menkes' kinky hair disease [6, 12, 20, 21] as same as brindled mouse (Mo^{br}) which is an allele at the X-linked *Mo* locus. The normal mice and heterozygotes ($Mo^{ml}/+$) have brownish black fur and mosaic brownish black and white fur, respectively, and develop normally [20]. However, its hemizygotes (Mo^{ml}/Y) and homozygotes (Mo^{ml}/Mo^{ml}) show white fur and curly whiskers from day 3 after birth, and ataxia and tonic seizure on day 8. They lose weight after day 10 and die with severe emaciation on around day 15. However, Mo^{ml}/Y and Mo^{ml}/Mo^{ml} mice given copper on day 7 can survive followed by normal growth and fertility. Mice that are not given copper have a copper deficiency in the brain and serum, and also have decreased serum ceruloplasmin levels as measured by copper oxidase activity. We also found that Mo^{ml}/Y mice which were not treated with copper showed atrophy in the thymus and spleen on day 14 [9].

Chimeric embryos have been valuable in the study of developmental mechanisms [19], sex determination [8], cell-cell interactions [14], and the rescue of otherwise lethal phenotypes [17]. We therefore produced chimeras between normal and macular mice in order to determine whether survival without copper treatment is possible, as well as to elucidate the mechanisms of the congenital

Accepted June 2, 1993
Received February 22, 1993
* Present address; Faculty of Pharmaceutical Sciences, Osaka University, Suita, Osaka 565, Japan.

copper deficiency in the brain and liver, but the copper accumulation in the kidney and intestine.

MATERIALS AND METHODS

Mice

Breeding pairs of C3Hf ($Mo^{ml}/+$) mutant and C3Hf ($+/Y$) mice were provided by the laboratory of Clinical Biochemistry, Wakayama Medical College. Mo^{ml}/Y (glucose phosphate isomerase-1 type B; GPI-1B) and Mo^{ml}/Mo^{ml} (GPI-1B) mice were obtained from mating $Mo^{ml}/+$ and Mo^{ml}/Y mice that were derived from our breeding colony. Mo^{ml}/Y and Mo^{ml}/Mo^{ml} were subcutaneously injected with 10 μg of Cu as cupric chloride per gram of body weight at 7 days after birth as described previously [18]. ICR, BALB/c (GPI-1A) and CDF1 mice (GPI-1A) were obtained from SLC, Inc., Shizuoka, Japan. Most mice were studied at 8 to 10 weeks of age.

Production of chimeras

Mo^{ml}/Mo^{ml} and Mo^{ml}/Y mice which were treated with $CuCl_2$ on day 7 were used. Embryos of Mo^{ml} mice were obtained from $Mo^{ml}/Mo^{ml} \times Mo^{ml}/Y$, and embryos of normal mice were obtained from BALB/c×BALB/c or CDF1× BALB/c. Female mice were superovulated by intraperitoneal injections of 7.5 i.u. of pregnant mare's serum, followed 48 hr later by an injection of 7.5 i.u. of human chorionic gonadotrophin. Female mice were paired overnight with male mice and vaginal plugs were checked the following morning. Two-cell-stage embryos were flushed with Hepes buffered Whitten's medium from the anterior portion of the uterine horns 24 hr later.

Detailed procedures of the production of chimeras by nuclear transplantation have been described previously [10]. Eggs were micromanipulated, washed several times and transferred to the oviducts of day 1 pseudopregnant ICR mice.

Analysis of GPI-1 isozymes

To examine the proportion of normal mouse cells to macular mouse cells, isozyme patterns of GPI-1 in the tissues were analyzed by a modification of the method of Eppig *et al.* [3]. GPI-1 isozymes were separated electrophoretically on a Titan III Zipzone cellulose acetate plate (Helena Laboratories) with 0.043 M Tris, 0.046 M glycine buffer (pH 8.5) for 2 hr at 180 V. Staining was carried out as described previously [10]. The proportions of GPI-1A and GPI-1B were determined from the original electrophoretic gels using a densitometer (Shimazu CS-910).

Chemical analysis

The copper levels in tissues were determined by flame atomic absorption spectrometry (Hitachi 208 type) after solubilization in HNO_3.

RESULTS

Table 1 shows the *in vitro* development of operated embryos when two normal strains were used. After microsurgery, experimental and control embryos were cultured for 4 days and the number of embryos that developed to the blastocyst stage was determined. Of 80 BALB/c↔Mo^{ml} chimeric eggs, 7 (9%) and 56 (70%) developed to morula and blastocyst stages, respectively and of 16 CDF1↔Mo^{ml} chimeric eggs, 7 (44%) and 5 (31%) developed to morula and blastocyst stages, respectively.

To produce the chimeric mouse, the operated embryos were transferred to the oviducts of day 1 pseudopregnant ICR mice. A total of 355 operated embryos were prepared, from which 52 live-born animals were obtained; 13 of the newborn animals were chimeric as determined by the coat color (Table 2). Eleven of these chimeras did not have discernible developmental abnormalities. However, 2 chimeras died at day 16 (chimera #1, male) and 22 (chimera #2, female) after birth, respectively. Figure 1 shows the normal, macular and chimeric mice. The fur was agouti in the $+/Y$ mouse, and white in the Mo^{ml}/Y mouse on day 14 after birth. Chimeras #1 and #2 had white and brown mosaic fur. These chimeras and the Mo^{ml}/Y mouse had curly whiskers, whereas the $+/Y$ mouse had straight whiskers. The body weight of the Mo^{ml}/Y mouse treated with $CuCl_2$ on day 7 gradually increased, although it weighed slightly less than that of the $+/Y$ as shown in Fig. 2. The Mo^{ml}/Y mice gained weight normally until about

TABLE 1. Development of chimeric eggs after nuclear transplantation *in vitro*

Type of egg	No. of cutured embryos	Developmental stage (%)		
		Arrested	Morula	Blastocyst
Unoperated				
BALB/c	77	14	8 (10%)	55 (71%)
CDF1	14	6	3 (21%)	5 (36%)
Mo^{ml}	197	18	29 (15%)	150 (76%)
Operated				
BALB/c↔BALB/c	21	9	1 (5%)	11 (52%)
$Mo^{ml}\leftrightarrow Mo^{ml}$	3	0	1 (33%)	2 (67%)
BALB/c↔Mo^{ml}	80	17	7 (9%)	56 (70%)
CDF1↔Mo^{ml}	16	4	7 (44%)	5 (31%)

Eggs were cultured for 4 days at 37°C in 5% CO_2, 5% O_2 and 90% N_2.

TABLE 2. Postimplantation development of chimeric eggs after nuclear transplantation

Type of eggs	No. of transferred embryo	No. of liveborn	Pups	
			liveborn (% of implants)	chimera (% of liveborn)
BALB/c↔Mo^{ml}	207	116	17 (15%)	2 (12%)
CDF1↔Mo^{ml}	148	84	35 (42%)	11 (31%)

Eggs were transferred into the oviduct of day 1 pseudopregnant recipient mouse.

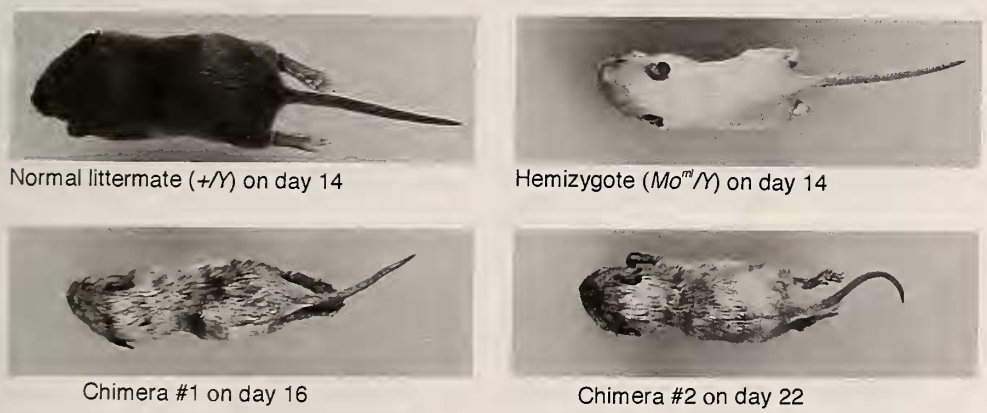
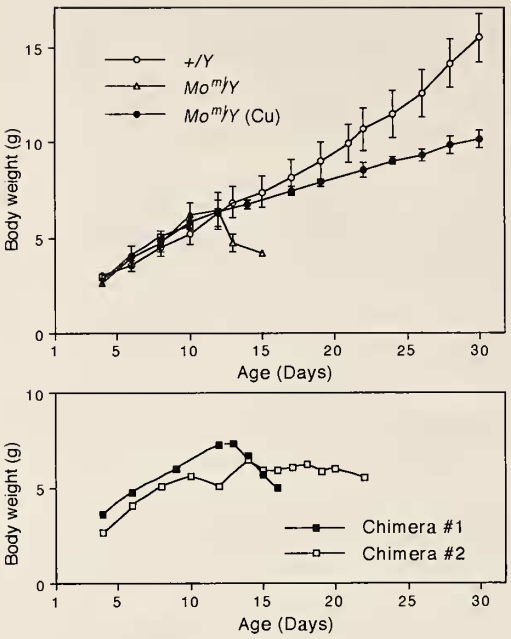


FIG. 1. Coat color distribution in chimeras #1 and #2.

day 10, but thereafter, showed gradual weight-loss and some physiological abnormalities such as frequent tonic seizure and ataxia, and died around day 15 in an emaciated condition (Fig. 2). The development of the Mo^{ml}/Mo^{ml} mice was similar to that of the Mo^{ml}/Y mice (data not shown). Iwane [5] also reported that the clinical features of the Mo^{ml}/Mo^{ml} mice were quite similar to those of the Mo^{ml}/Y mice. The body weight of chimera #1, which was produced between BALB/c and macular mice, gradually increased until day 12. Thereafter, it gradually lost weight, had frequent tonic seizures and ataxia like the Mo^{ml}/Y mouse and died with severe emaciation on day 16.



Chimera #2, which was produced between CDF1 and macular mice, also gained weight normally until about day 14, when it reached a plateau and thereafter slightly lost weight. This mouse showed occasional tonic seizures and ataxia and died with severe emaciation on day 22.

Figure 3 shows the distribution of chimerism among the various tissues, as determined by GPI-1 activity, in 4 chimeras. The proportion of BALB/c to macular mouse cells in the tissue of chimera #1 ranged from 45:55 to 20:80 except for the liver. In the chimera #2, the liver, thymus, lung and spleen were composed only of macular mouse cells and the ratio of CDF1 to macular mouse cells in

FIG. 2. The body weight gain of normal (+/Y), macular (Mo^{ml}) and chimeric mice. Mo^{ml} (Cu); Mo^{ml} mouse was treated with 10 μg of copper per gram of body weight at 7 days after birth. Chimera #1 died 16 days and chimaera #2 died 22 days after birth.

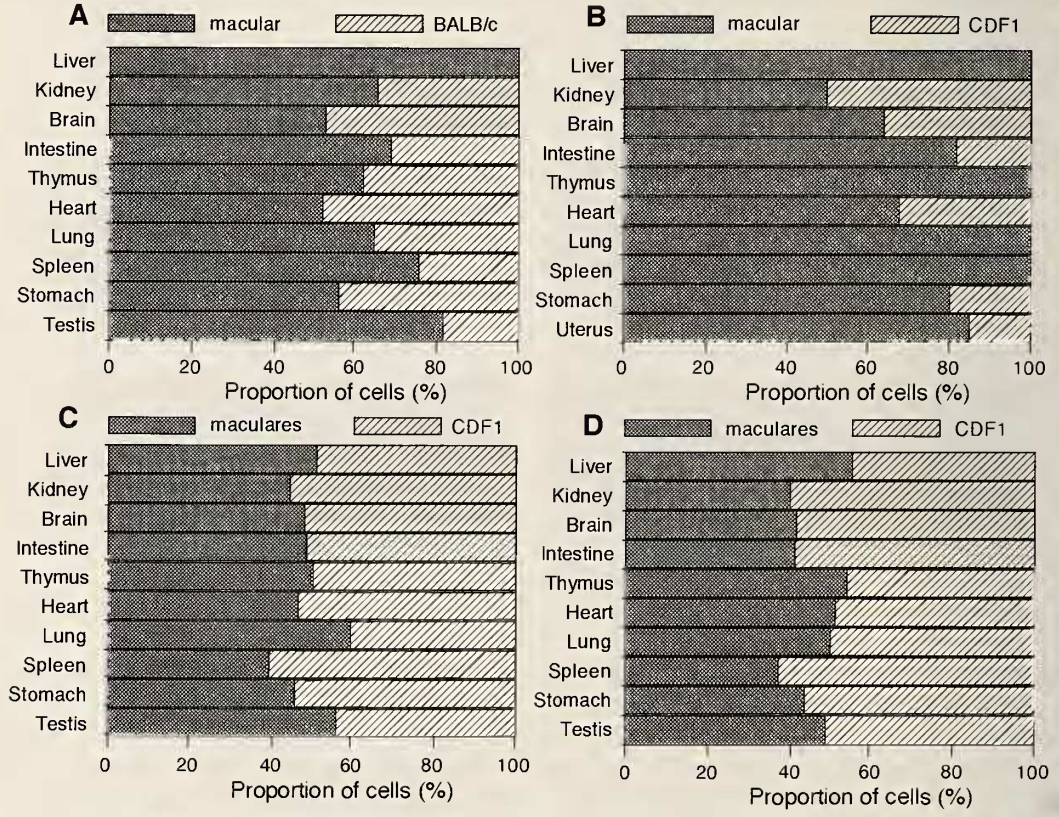


FIG. 3. Distribution of cells in chimeric mice. The proportion of each type of cell was determined from the relative levels of GPI-1 isozymes. The GPI-1 isozyme of BALB/c and CDF1 mouse is the AA type and that of the macular mouse is the BB type. A, chimera #1; B, chimera #2; C, chimera #3; D, chimera #4.

other tissues ranged from 80:20 to 50:50. In the surviving chimeras (#3 and #4), normal mouse cells were present in all tissues including the liver. The other surviving chimeras were also made of normal and macular mouse cells in all tissues (data not shown).

Table 3 shows the relative organ weight of chimeras. We previously reported that Mo^{ml}/Y mice had atrophy in thymus and spleen tissues [9]. The relative organ weight of Mo^{ml}/Y mice treated with Cu on day 7 did not differ from those of $+/Y$, but, in the Mo^{ml}/Y mice which were not treated with Cu, the relative weights of the spleen and thymus were lower, while those of the brain, kidney, heart and lung were higher than in the $+/Y$ mice at day 14. Chimera #1 had enlarged brain,

Table 3. Relative organ weight of Mo^{ml} mouse at day-14 and chimeric mouse

	Liver	Kidney	Brain	Heart	Lung	Spleen	Thymus
$+/Y$	3.24±0.39	1.21±0.15	4.27±0.45	0.51±0.06	1.15±0.14	0.47±0.08	0.44±0.10
$Mo^{ml}/+$	3.27±0.21	1.25±0.13	5.02±0.61	0.59±0.09	1.32±0.14	0.53±0.15	0.49±0.10
Mo^{ml}/Y	3.16±0.51	1.54±0.12*	7.10±0.97*	0.83±0.11*	1.44±0.11*	0.19±0.04*	0.28±0.09*
Mo^{ml}/Y (Cu)[a]	3.52±0.58	1.34±0.17	4.98±1.16	0.59±0.15	1.21±0.22	0.49±0.15	0.42±0.11
#1 Chimera	4.02	2.24	7.92	1.32	2.04	0.42	0.36
#2 Chimera	3.70	1.97	7.99	0.84	1.54	0.22	0.11

The value is organ weight/body weight (%).
[a]; Mo^{ml} mouse was treated with 10 μg of copper per gram of body weight at 7 days after birth
*; Significantly different from normal mice ($+/Y$), $p<0.01$.

Table 4. Copper concentration in organs of various mouse

Mouse	Concentration of Cu (μg/g tissue), Mean±S.D.			
	Liver	Brain	Kidney	Intestine
Day 14 after birth				
BALB/c (male)	39.2±7.6	1.8±0.1	2.5±0.2	2.6±0.6
BALB/c (female)	40.4±9.4	1.8±0.2	2.4±0.1	2.2±0.1
CDF1 (male)	33.6±7.3	1.7±0.1	2.4±0.2	2.1±0.2
CDF1 (female)	38.1±10.4	1.8±0.2	2.4±0.1	2.2±0.1
$+/Y$	20.3±7.6	1.4±0.3	2.5±0.3	3.1±0.3
$Mo^{ml}/+$	4.2±0.6	0.8±0.1	13.8±4.0	10.1±4.9
Mo^{ml}/Y	3.3±0.3	0.4±0.1	11.4±2.5	15.5±7.4
Mo^{ml}/Mo^{ml}	3.2±0.2	0.3±0.1	9.4±0.8	16.3±4.3
Mo^{ml}/Y (Cu)[a]	5.0±0.6	0.7±0.1	31.8±2.3	16.1±1.5
Mo^{ml}/Mo^{ml} (Cu)[a]	4.2±0.7	0.7±0.1	27.3±4.5	18.4±5.5
Chimera #1	3.6	0.4	11.9	2.5
Chimera #2	3.5	0.9	13.9	4.1
Adult				
$+/Y$	4.1±1.1	5.3±1.0	4.2±0.4	1.6±0.2
Mo^{ml}/Y (Cu)[a]	3.8±0.7	2.5±0.4	18.4±4.5	2.4±0.5
$Mo^{ml}/+$	4.7±0.7	3.5±1.3	27.8±8.2	3.6±0.7
Chimera #3	5.8	5.0	12.4	3.0
Chimera #4	5.2	4.9	28.3	2.6

[a]; Macular mouse was treated with 10 μg of copper per gram of body weight at 7 days after birth. Chimeras #1 and #2 died 16 and 22 days after birth, respectively. The copper concentration was measured as described in Materials and Methods.

kidney, heart and lung, but did not show atrophies in thymus and spleen tissues. On the other hand, chimera #2, like the Mo^{ml}/Y mouse, had a smaller spleen and thymus, and enlarged brain, kidney, heart and lung compared with those of $+/Y$ mice at day 14.

Table 4 shows the copper concentration in the tissues of various strains of mice. In macular mice at 14 days after birth, the copper concentration in the liver and the brain were significantly low, whereas those in the kidney and intestine were remarkably high. In macular mice treated with copper, the copper concentration in the brain was intermediate between those of normal and macular mice, but the level in the kidney and intestine was extremely high, and that in the liver was also extremely low in macular mice. In the $Mo^{ml}/+$ mice, the copper concentration in the liver and the brain was equal to that of macular mice treated with copper; that in the kidney was similar to that of macular mice, and in the intestine, the copper concentration was intermediate between normal and macular mice. The copper level of chimera #1 remained low in the liver and the brain and high in the kidney. However, the copper level in the intestine was the same as that in the normal mouse. Chimera #2 had a low and high copper levels in the liver and kidney, respectively. The copper level in the brain and intestine were similar to those in the normal mouse.

During the development of normal mice, the copper concentration in the brain and kidney increased, while those in the liver and intestine decreased, especially at a higher rate in the liver. In the adult mouse, the copper level of Mo^{ml}/Y (Cu) remained low in the brain and high in the kidney and intestine. The copper level of Mo^{ml}/Y (Cu) in the liver was the same as that of the normal mouse. In the adult $Mo^{ml}/+$ mice, the copper concentration in the liver was equal to that of normal mice, but in the brain, it was intermediate between $+/Y$ and Mo^{ml}/Y (Cu) mice. Furthermore, in the kidney and intestine, it was as high as that in Mo^{ml}/Y (Cu) mice. The copper level of chimeras #3 and #4 remained high in the kidney and intestine. However, the copper levels in the liver and the brain were same as those of normal mice.

DISCUSSION

The macular mutant mouse is considered to be a model of Menkes' kinky hair disease because of its clinical, biochemical and pathological similarities to the disease [7, 12, 20]. The genetic lesion established the existence of a specific transport system for copper, but the problem has not been solved thoroughly.

The utility of the genetic approach is strengthened by experimental procedures that permit the close intermingling of mutant and normal cells in various tissues. We used nuclear transplantation to produce chimeras between normal (BALB/c and CDF1) and macular mice in an attempt to produce animal with macular mouse cells distributed in various tissues. We produced 13 chimeric mice. All Mo^{ml}/Y and Mo^{ml}/Mo^{ml} mice which were not treated with copper normally gained weight until about day 10, but then ceased to grow, gradually becoming inactive and dying on about day 15. However, the majority of normal $\leftrightarrow Mo^{ml}$ chimeras grew normally without copper treatment even after day 10. Furthermore, surviving chimeras did not show any tonic seizures and ataxia, although chimeras #1 and #2 died at day 16 and 22 after birth, respectively. In 13 liveborn chimeras, all contained a component from Mo^{ml} hemizygotes or homozygotes. However, examination of the tissue distribution of macular mouse cells in the dead chimeras revealed dramatic differences compared to the other surviving chimeras. In surviving chimeras (#3 and #4), normal mouse cells were contained at a ratio of 40–60% in all tissues. However, the macular mouse cells comprised above 99% of the liver in chimeras #1 and #2, although the thymus, lung and spleen in chimera #2 were also composed only of macular mouse cells. The effects of the Mo^{ml} gene could not be rescued in any chimeric environment in the liver. Thus, the liver is one of the "critical tissues" in which the presence of a high proportion of macular mouse cells leads to an abnormal, nonviable phenotype.

The thymus and spleen were smaller in macular mutant mice. Brindled mice, in contrast, show little effect on thymus weight, whereas an atrophic effect is evident in the spleen [15]. In copper

deficient mice, the larger spleen and smaller thymus are characteristic [16]. Atrophy of both thymus and spleen is characteristic of macular mutant mice. Chimera #2 which had no normal mouse cells in the thymus and spleen showed the atrophy of these tissues similar to that of macular mutant mice. However, the thymus and spleen weight was little affected in chimera #1 even though macular mouse cells occupied 60 and 75% of these organs, respectively. Therefore, it seems that the abnormal development of the thymus and spleen in the macular mouse can be largely corrected when macular mouse cells in these tissues are allowed to develop in conjunction with normal thymocytes and spleen cells.

There were low levels of copper in the brain and liver, and high levels in the kidney and intestine of macular mice. The disturbance of copper homeostasis in Mo^{ml}/Y mice agreed closely with those reported by Ooyama *et al.* [13] and Tanaka *et al.* [18]. Copper levels of brindled mice are low in the brain and liver, but high in the kidney and intestine [15]. In the $Mo^{ml}/+$ mice, the level of copper concentration in the brain and intestine was intermediate between normal and macular mice, but that in the liver was extremely low. The copper level in the kidney was also higher than that in normal mice. As a result of random X-inactivation, $Mo^{ml}/+$ mice are genetic mosaics containing an approximately equal number of normal and macular cells. Chimeras #1 and #2 were mosaics with intermingling normal and macular cells in various tissues except the liver. Thus, the copper concentration in the livers of chimeras #1 and #2 were expected to be similar to those of Mo^{ml}/Y or Mo^{ml}/Mo^{ml}, while those in the brain, kidney and intestine should be the same as those of $Mo^{ml}/+$. However, the copper concentrations in the intestines of chimeras #1 and #2 were similar to that in the $+/Y$ intestine, whereas the copper concentration in the brain of chimera #1 was the same as that of the Mo^{ml}/Y. The copper concentrations in the liver of Mo^{ml} mice and chimeras #1 and #2 were extremely low in comparison with those of normal mice. However, the copper concentrations in the liver of surviving chimeras (#3 and #4) and adult Mo^{ml}/Y (Cu) mice were the same as that of the normal mouse. In surviving chimeras, it can be considered that the disturbance of copper homeostasis in macular mouse cells was directly improved by normal mouse cells or copper homeostasis at tissues level was maintained by normal mouse cells. Nevertheless, the copper levels in the liver of surviving chimeras at day 14 after birth remains low. Further investigation is required to elucidate a connection between the copper content and the ratio of chimerism in the liver at day 14 after birth. Those of the kidneys in chimeras #1 and #2, and the severity of the changes in the copper content was unexpected considering the intestine copper levels were equal to those in normal mice. Copper content in the intestine was unexpected since the brain levels were similar to those of $Mo^{ml}/+$ mice. In surviving chimeras, it may be considered that the disturbance of copper homeostasis in macular mouse cells was directly improved by normal mouse cells or that copper homeostasis at the tissue level was maintained by normal mouse cells.

REFERENCES

1 Danks DM (1977) Copper transport and utilization in Menkes' syndrome and in mottled mice. Inorg Perspect Biol Med 1: 73–100

2 Danks DM, Campbell PE, Stevens BJ, Mayne V, Cartwright E (1972) Menkes' kinky hair syndrome. An inherited defect in copper absorption with widespread effects. Pediatrics 50: 188–201

3 Eppig JJ, LP Kozak, EM Eicher, LC Stevens (1977) Ovarian teratomas in mice are derived from oocytes that have completed the first meiotic division. Nature 269: 517–518

4 Holzman NA (1976) Menkes' kinky hair syndrome: A genetics disease involving copper. Fed Proc 35: 2276–2280

5 Iwane S, Kawasaki H, Yamano T, Shimada M (1989) Golgi study on the homozygote (ml/ml) of macular mutant mouse. Brain Dev 11: 151–160

6 Kawasaki H, Shimada M, Yamano T, Nishimura M (1987) Golgi study on brain of macular mutant mouse as a model of Menkes kinky hair disease. Acta Neuropathol (Berl) 72: 349–54

7 Kawasaki H, Yamano T, Iwane S, Shimada M (1988) Golgi study on macular mutant mouse after copper therapy. Acta Neuropathol (Berl) 76: 606–612

8 McLaren A (1975) Sex chimaerism and germ cell distribution in a series of chimaeric mice. J Embryol Exp Morphol 33: 205–216

9 Nakagawa S, Fukata Y, Nagata H, Miyake M, Takao H (1993) The decreased immune responses in macular mouse, a model of Menkes' kinky hair disease. Res Commun Chem Patho Pharmacol 79: 61–73

10 Nakagawa S, Sumita K, Hama T, Mayumi T (1993) Production of Chimeric Mice by Exchanging Nuclei from Blastomeres of the Two-Cell Embryo. Zool Sci 10: 645–651

11 Nishimura M (1975) A new mutant mouse, macular(Ml) (in Japanese). Exp Anim (Tokyo) 24: 185 (in Japanese)

12 Onaga A, Kawasaki H, Yamano T, Shimada M, Nishimura M (1987) Light and electron microscopic study on cerebellar cortex of macular mutant mouse as a model of Menkes kinky hair disease. Brain Dev (Tokyo) 9: 265–9

13 Ooyama H, Kimura M, Samukawa A, Higa K, Matsumura Y, Nishimura M (1975) A biochemical study of a C3H mutant strain (Ml/y). Seikagaku 51: 845 (in Japanese)

14 Papaiannou VE, K Artzt, GB Dooher, D Benner (1979) Effects of chimerism for two T/t complex mutations on sperm function. Gamete Res 2: 147–151

15 Prohaska JR (1983) Changes in tissue growth, concentrations of copper, iron, cytochrome oxidase and superoxide dismutase subsequent to dietary or genetic copper deficiency in mice. J Nutr 113: 2148–2158

16 Prohaska JR, Lukasewycz OA (1989) Copper deficiency during perinatal development: Effects on the immune response of mice. J Nutr 119: 922–931

17 Stevens CL (1978) Totipotent cells of parthenogenetic origin in a chimaeric mouse. Nature 276: 266–267

18 Tanaka K, Kobayashi K, Fujita Y, Fukuhara C, Onosaka S, Min K (1990) Effects of chelators on copper therapy of macular mouse, A model animal of menkes' kinky disease. Res Commun Chem Path Pharmacol 69: 217–227

19 West JD (1984) Cell markers. In "Chimeras in developmental biology" Eds by Le Douarin N, McLared A, Academic Press, London, pp 39–67

20 Yamano T, Shimada M, Kawasaki H, Onaga A, Nishimura M (1987) Clinico-pathological study on macular mutant mouse. Acta Neuropathol (Berl) 72: 256–260

21 Yamano T, Shimada M, Onaga A, Kawasaki H, Iwane S, Nishimura M (1988) Electron microscopic study on brain of macular mutant mouse after copper therapy. Acta Neuropathol (Berl) 76: 574–80